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Original article

Probiotic bacteria and/or Bile Salts Potential Therapeutics of Induced Ulcerative Colitis

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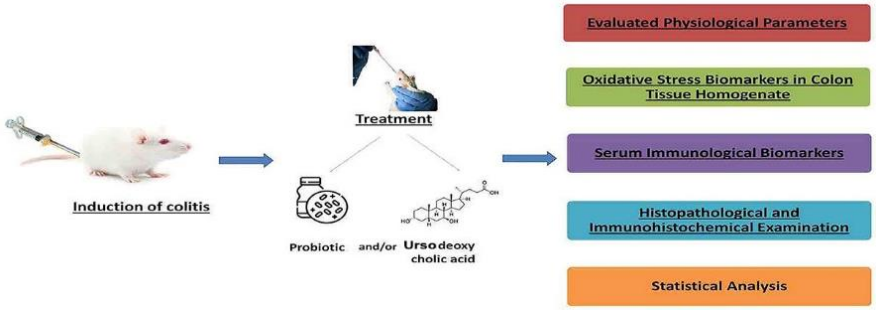
ABSTRACT

Ulcerative colitis (UC) is a common chronic inflammatory bowel disease characterized by the upregulation of oxidative stress and pro-inflammatory cytokines. Probiotic bacteria and Ursodeoxycholic acid (UDCA) have been suggested as promising choices for the treatment of UC. The purpose of the current study was to assess the impact of probiotics and/or UDCA on acetic acid-induced colitis in rats. Rats were randomly categorized into seven groups: normal control, UDCA, probiotic, acute colitis, acute colitis+probiotic, acute colitis+UDCA, and acute colitis+Probiotic+UDCA. UDCA (10mg/kg/day) or probiotic 0.5 mg/kg/day) was orally administered for 7days after24hours of induction of ulcerative colitis by 4% acetic acid. The rats were euthanized 24h after the last dose of treatment and on the 8th day post acetic acid instillation. Clear serum was separated for estimating immunological parameters as well as colon sections were evaluated for macroscopical, histopathological and oxidative stress. Treatment with probiotics and or UDCA significantly reduced disease index, wet/dry colon weight ratio, ulcer area, and macroscopic disease scores, with some histopathological changes. Probiotics and/or UDCA also effectively reduced the levels of myeloperoxidase and malondialdehyde (90.6%,75.9%,84.6%) and increased glutathione levels (5.5%,39.4%,45.6%). Regarding the inflammatory markers, probiotics and/or UDCA reduced significantly pro-inflammatory cytokine levels such as interleukin-17 and 6, and C reactive protein, associated with increases in anti-inflammatory cytokine IL10. According to our findings, oral administration of probiotics and/or UDCA ameliorates acetic acid-induced colitis in rats via decreasing oxidative stress and modulating the pro-inflammatory cytokine levels and MPO activity. Therefore, these treatments may be an effective therapeutic candidate for ulcerative colitis treatment.

Graphical abstract



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1. Introduction

Inflammatory bowel disease (IBD) is an inflammatory disease of both the small and large intestine [1]. It is categorized into Crohn's disease (CD), ulcerative colitis (UC), and indeterminate or unclassifiable colitis(IC). These conditions usually share common characteristics such as melena, abdominal pain, diarrhea, fever, weight loss, and fatigue; each has unique features[2].

Ulcerative colitis(UC) is a chronic inflammatory condition affecting colonic mucosa. It starts in the rectum and extends to the colon[3]. Ulcerative colitis is associated with abscesses, strictures, and fistulas. Histologically it shows superficial mucosal and submucosal inflammatory changes with cryptitis and crypt abscesses[4]**,** edema, abnormal tissue architecture, ulcerations and goblet cell mucus depletion[5]**.** Although the etiology is still unknown, ulcerative colitis appears to be caused by a combination of environmental and hereditary factors[6]. Ulcerative colitis is characterized by overproduction of pro-inflammatory cytokines, oxidative stress[7]**,** and increased intestinal permeability[2].

Anti-inflammatory and immunosuppressive medications, antibiotics, as well as surgical procedures are the basic of UC therapies [8]. Although these therapies lessen the disease's inflammatory activity and symptoms, they are not curative and can lead to serious side effects in patients [9]**.** Hence, great attention has been given to find treatments with less side effects. Probiotic bacteria have been proposed as a possible option for UC therapy[10].

Probiotics are live microbial dietary supplements that benefit the host by improving the microbial balance in the intestine[11]. Probiotics decrease pro-inflammatory cytokine production(e.g.,TNF-α and IL-1β)and elevate the production of anti-inflammatory cytokines(such as IL-10)[12]. Among the different species of bacteria that are used as probiotics is probiotics composed of *Ruminococcus flavefaciens. Ruminococcus* is a genus of bacteria belonging to phylum Firmicutes and class Clostridia. *Ruminococcus flavefaciens* is an anaerobic streptococcus organism isolated from a bovine rumen and is considered one of the dominant species of cellulose-decomposing bacteria in the rumen[13].

Bile acids are the byproducts of cholesterol metabolism in vertebrates. They are synthesized by hepatocytes as primary bile acids. These primary bile acids are conjugated after being synthesized to either taurine or glycine to give the bile salts. However, the secondary bile acids result from bacterial actions in the colon[14,15]**.**

Ursodeoxycholic acid(UDCA) was first isolated from bear bile; it has been used as a remedy in traditional Chinese medicine for many years to treat many different diseases. UDCA occurs naturally at low levels in human bile[16]. The biological actions of UDCA include immunomodulatory and anti-apoptotic actions[17].

In this work, we sought to assess the preventive effects of probiotics and/or UDCA against acetic acid-induced UC in rats, as well as their impact on colonic oxidative stress and certain mediators of the inflammatory response.

2. Materials and methods

2.1. Experimental design

Forty-eight adult male albino rats weighing 150-200 g(4-6 weeks) were used in the present study.The animals were housed in clean cages and had free access to food and water(*ad libitum)*. They were maintained at21–24˚C and 40–60% relative humidity with a 12 h light-dark cycle. All animal procedures were performed in accordance with National Organization For Drug and Central Ethics Committee(NODCA Ethics Committee acceptance No. NODCAR/1/32//20222.

Rats were randomly allocated into 7 groups.
Normal control group(n:6), instilled physiological saline; UDCA treated group (n: 6),rats received10 mg/kg of UDCA(Ursofalk ®Capsules, under license of Dr. Falk- Germany, MINAPHARM- Egypt), orally for 7days; Probiotic treated group(n:6),rats received 0.5 ml/kg of probiotics(probiotic suspension produced by Bactizad, Heliopolis,Cairo,Egypt), orally for 7 days; UC group(n:8),rectal enema was used to induced colitis with 1 ml of 4% acetic acid (ADWIC, Egypt) [18];UC-UDCA treated group(n:8),rats were instilled with 1ml of 4%acetic acid enema followed by administration of 10mg/kg of UDCA orally for 7days; UC- Probiotic treated group(n:8),rats were instilled with 1ml of 4%acetic acid enema followed by administration of 0.5ml/kg of probiotic orally for 7days; and UC-UDCA- Probiotic treated group(n:8), rats were instilled with 1ml of 4%acetic acid followed by administration of probiotic and UDCA orally for 7 days[19,20]**.**

2.2. Tissue collection and preparation

Body weights were measured in all animals at the beginning and the end of the study. 24 hours after the last dose of medication, on the 8thday post acetic acid instillation, stool consistency and color were inspected and recorded for each rat. Blood samples were obtained in dry, clean centrifuge tubes after the rats were euthanized by cervical dislocation. Blood was centrifuged for 10 minutes at 3000 rpm (MPw-350, Warsaw, Poland) after being allowed to clot at room temperature. Clear serum was separated and stored at - 80 °C for estimating the CRP titer (bioMérieux, Egypt), and IL-10 and IL-17 levels by ELISA (NOVA, China). A 10 cm section of the distal colon was removed, separated longitudinally from the adhering adipose tissue, rinsed with saline to eliminate faecal remnants, and weighed. The colons were photographed and assessed for

macroscopic damage scoring as Disease Activity Index (DAI) scores. Degree of bleeding, weight loss and stool consistency are known as colon macroscopic scoring, according to (Vasina *et al.*, 2010)[21].

The colon was divided into three portions; the1st was fixed in10%buffered formol saline for the histopathological assessment and immunohistochemical analysis using Interleukin6(IL-6) Monoclonal Antibody(AMC0864,Thermo Fisher Scientific;Fremont,USA). The remaining 2 parts; 1 cm in length were weighed and used for wet/dry ratio; the other part was homogenized and the supernatant was used for the assay of MDA according to the method of Deniz *et al.* (1997)(Biodiagnostic,Egypt)[22] and GSH according to the method described by Beutler *et al*. (1963)(Biodiagnostic,Egypt)[23] and MPO activity was evaluated according toBradley *et al*.(1982)(Biodiagnostic,Egypt)[24].

2.3. Statistical Analysis

A one-way analysis of variance (ANOVA) and Tuky-post Kramer's hoc multiple comparison test were used to evaluate the data. Using the software program Instate version 3, all statistical analyses were carried out. Graphs were created on a computer using the GraphPad prism application(GraphPad Software Inc. V5, San Diego, CA, USA).At a level of P<0.05, statistical significance was recognized.

3. Results

3.1. Physiological parameters and body weight changes: -

Body weight changes compared to UC group, UDCA significantly($p<0.05$) increased body weight either alone or in combination with probiotics however probiotics alone could increase rat BW. The colon wet-dry ratio showed significant($p<0.05$) improvement after treatment with UDCA, probiotics, or both when compared to the UC group. The ulcerative colitis group showed a significant decrease in colon length as compared with normal control rats($p<0.05$). Treatment with UDCA, probiotics, or both; significantly($p<0.05$) increased colon length in comparison to the UC group. The best improvement was observed in the combined treated group followed by UDCA and probiotic-treated groups respectively. UDCA and /or probiotic oral administration significantly reduced the number of colon ulcers as compared to UC treated group($p< 0.05$) **(Table 1).**

3.2. Colon macroscopic examination and macroscopic damage scoring:

Gross examination of colons of the UCgroup showed extensive tissue necrosis, visible erosions, and severe hemorrhage. UDCA, probiotic or combined administration, protected against mucosal damage and tissue necrosis as shown in Fig(1).

3.3. Oxidative stress parameters

Colon homogenate content of MDA and MPO increased significantly($p>0.05$) in comparison to normal rats. Administration of UDCA and/or probiotic treatment improved colon ulcerative colitis oxidative stress. MDA content significantly($p>0.05$) decreased in UDCA, combined therapy, and probiotic-treated rat groups by 90.6%, 84.6%, and 75.9%, respectively. PO activity also showed a reduction (1.41± 0.17),(1.61± 0.54)and(3.63± 1.56)in UDCA, probiotic, and combined therapy treated groups respectively.

In the UC group, GSH content decreased significantly ($p < 0.05$) in comparison to normal control rats. The combined therapy of UDCA and probiotic followed by probiotic treatment and UDCA treatment alone increased GSH by 45.6%,39.4%,and5.5% respectively; Fig.(2).

Groups	Body weight change(g)	Colon length(cm)	Number of colon ulcers	Colon relative weight(mg/g body weight)	Colon wet dry weight ratio(W/D Ratio)
Normal control	5.38 ± 0.78*	18.95± 0.41*	0.0 ±0.0*	5.87±0.29*	3.9± 0.23*
UDCA	43.67± 4.3#*	18.20± 0.63*	0.16 ±0.16*	7.09 ±0.24	4.25± 0.10*
Probiotic	21.33± 1.5#	16.98 ±0.37*	0.0 ±0.0*	7.25±0.11	4.06± 0.13*
UC	18.25± 10.7#	15.06± 0.47#	4.12±1.2#	9.21±1.09#	5.34± 0.21#
UC- UDCA	37.5 ± 4.26#*	16.58±0.59#*	1.62± 0.49*	6.64±0.42	3.98 ±0.17*#
UC-Probiotic	9.75 ± 4.21#*	15.16±0.62#*+	0.50± 0.18*	7.6±0.78	3.98 ±0.16*#
UC- UDCA – Probiotic	28.0 ± 4.50#	17.1± 0.76*	0.62± 0.18*	7.65±0.41	4.35± 0.11*

Table(1): Effect of Ursodeoxycholic acid or/and probiotic treatment on body weight change, colon length, number of colon ulcers, colon relative weight, and colon wet/dry weight ratio in rats with ulcerative colitis induced by acetic acid.

3.4. *Immunological parameters*

Proinflammatory markers(serumIL17and-CRP) increased significantly($p>0.05$)in the UC group in comparison to normal rats. UDCA alone or in combination with probiotics and probiotic oral administration decreased serumIL-17(2.19±0.33, 8.18 ±1.84,and69.28±12.21 respectively) and CRP significantly in comparison to UC group($p<0.05$).

While the serum level of the anti-inflammatory cytokine;IL-10 showed a significant decrease in the UC group in comparison to the normal control group at $p<0.05$. Treatment with UDCA alone or in combination with probiotics significantly increased the level of serumIL-10 respectively($p<0.05$); while a non-significant increase was noticed in oral administered probiotics in ulcerative colitis-induced rats Fig.(3).

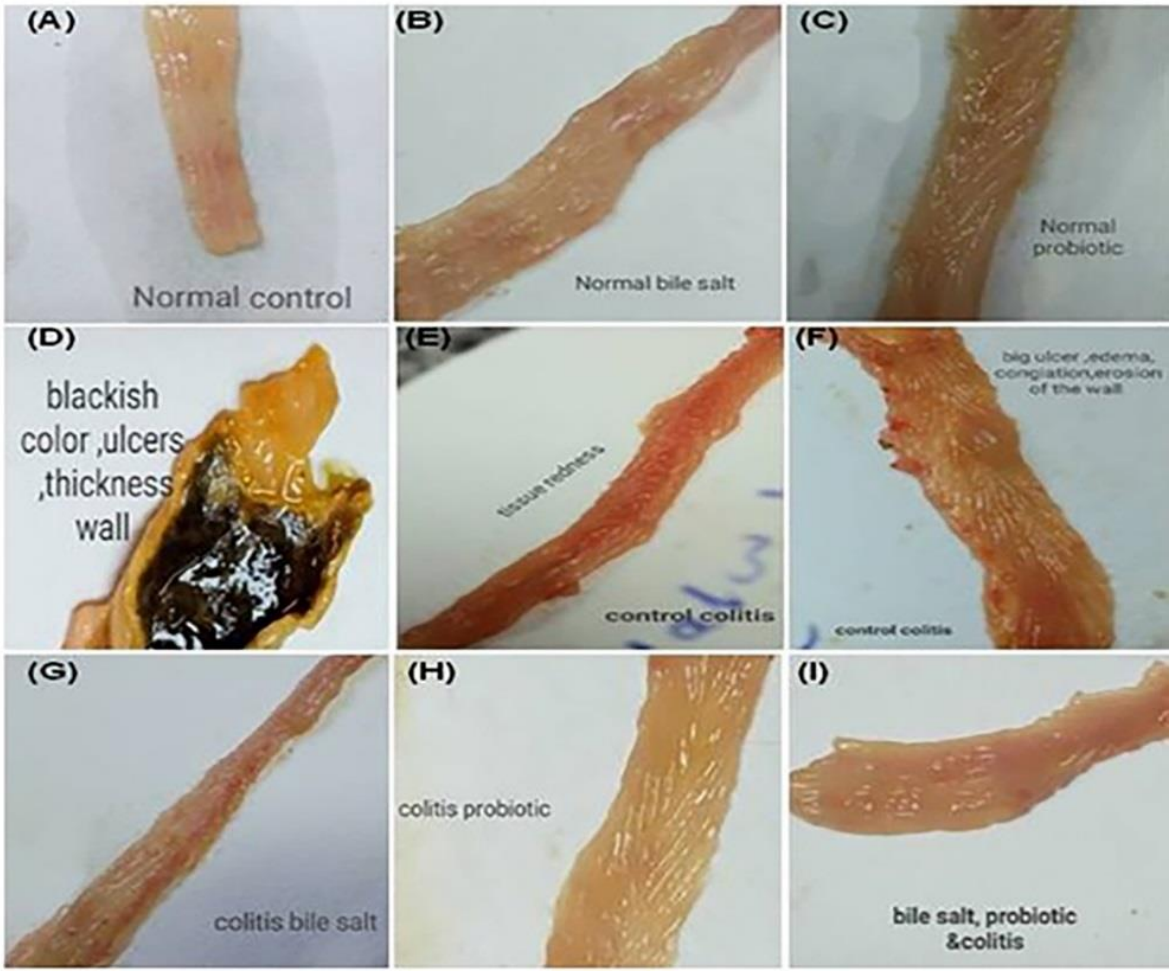

Fig.(1): Macroscopic examination of rat colon in different groups.(A)naive control, (B)ursodeoxycholic acid,(C)probiotic,(D,E&F)ulcerative colitis tissue necrosis, wide surface area with severe haemorrhage, mucosal lining damage with visible erosions and ulcers,(G)ulcerative colitis treated with ursodeoxycholic acid showing slight hyperemic rat colon,(H)ulcerative colitis treated with probiotic showing normal appearance,(I)ulcerative colitis treated with ursodeoxycholic acid combined with probiotic revealing slightly hyperemic mucosa, with no ulcer or erosion.

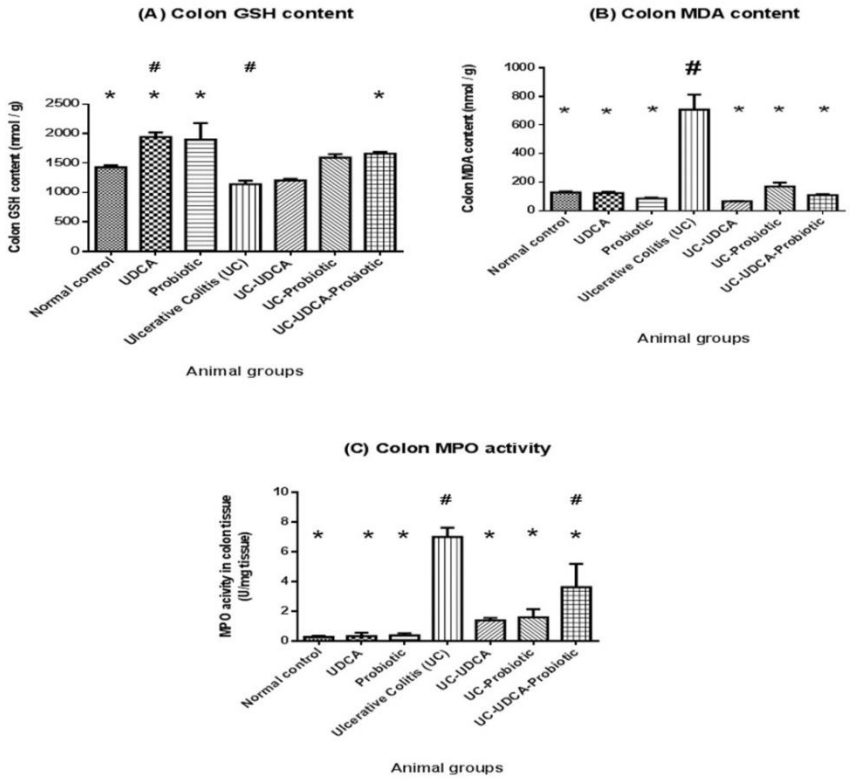

Fig.(2): Oxidative stress markers of different groups.**(**A):Colon GSH content,(B):Colon MDA content, and(C):Colon MPO content as compared with rats subjected to acetic acid-induced ulcerative colitis.# Significant difference from control gp at p<0.05. * Significant difference from ulcerative colitis gp at p<0.05. + Significant atP<0.05 compared with Ulcerative Colitis-Ursodeoxycholicacid -Probiotic group.

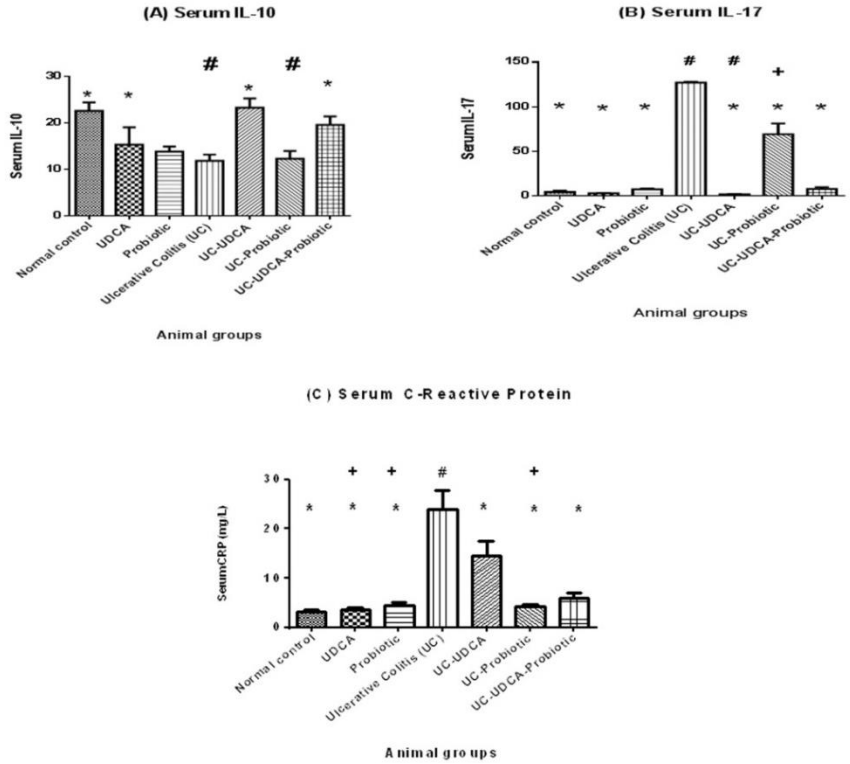

Fig.(3): Inflammatory markers of different groups.**(**A):Serum IL-10,(B):Serum IL-17 and(C):Serum C-reactive Protein(CRP) as compared with rats subjected to acetic acid-induced ulcerative colitis. Significant difference from control gp at p < 0.05.*Significant difference from ulcerative colitis gp at p<0.05.+Significant atP<0.05 compared with Ulcerative Colitis-Ursodeoxycholicacid -Probiotic group.

3.5. *Histopathological examination*

Acetic acid administration induced extensive pathological changes where the colonic mucosa appeared with distorted crypts, epithelial cell hyperplasia with loss of goblet cells, and severely ulcerated surface epithelium. Diffuse inflammatory cell aggregates were noticed in mucosal, submucosal, and muscularis layers as well as congestion, blood vessel dilation, and stromal edema in the submucosa were noticed. Histopathological examination of colon specimens of UDCA treated group showed mostly normal colonic tissue with focal lymphoid aggregates. Remarkable improvement could be seen after treatment of the colitis group with probiotics alone or in combination where colon tissue looked comparable to control Fig.(4).

3.6. *Immunohistochemical examination*

By observing immunohistochemistry sections, IL-6 was found mainly distributed in the colon mucosa and submucosa. In the UC group, IL-6 protein expressions were considerably higher ($P<0.05$) than normal control. Conversely, immunoexpression of IL-6 in both UDCA or /and probiotic; was significantly lower than the ulcerative colitis group at($P<0.05$) Fig.(5).

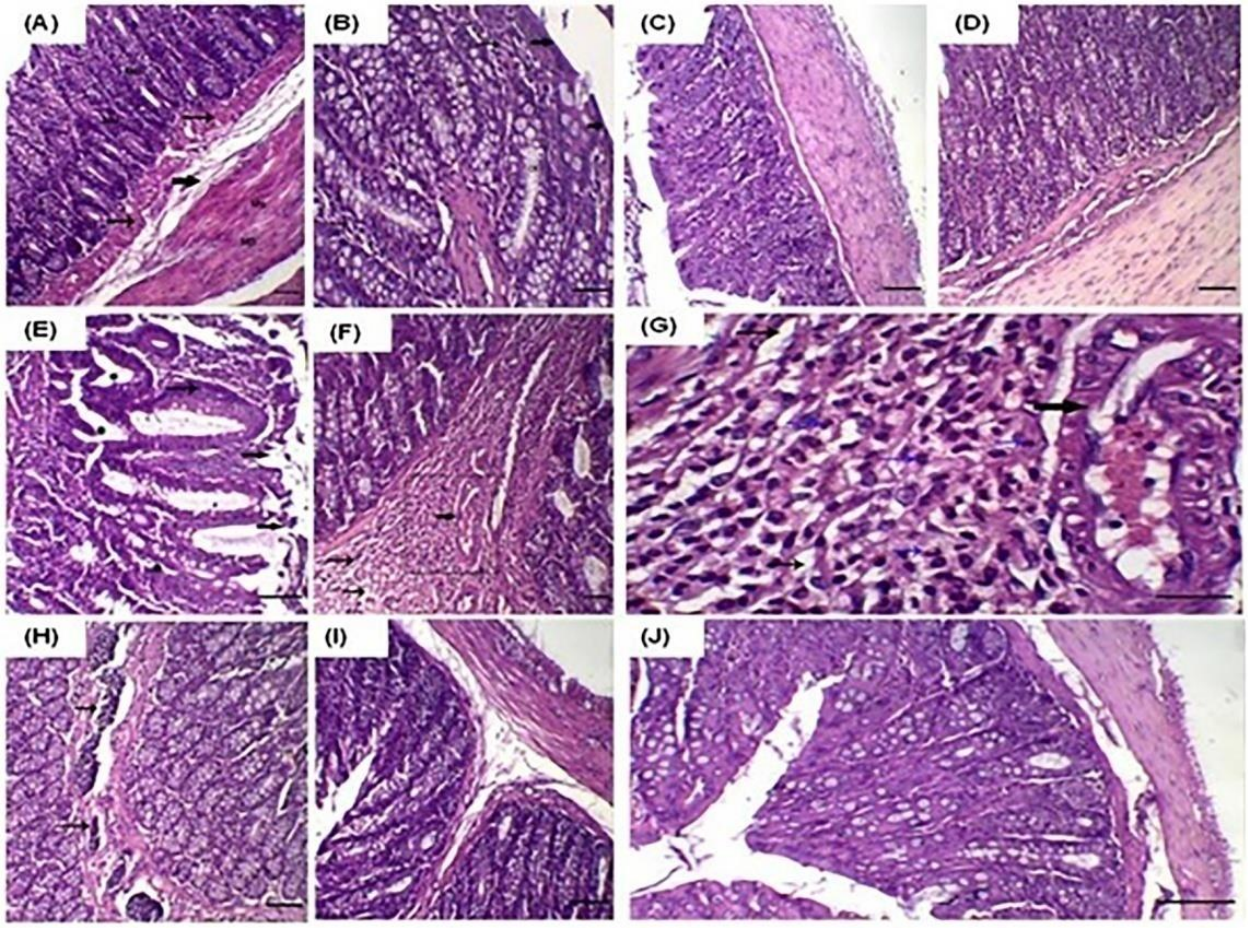

Fig. (4): Photomicrograph of colon tissues from: Control group(A):showing mucosa(MU), muscularis mucosa(thin arrow), submucosa(thick arrow), muscularis submucosa(MS)(H&E, X:100); (B):crypt(CR), goblet cell(G),mucosal epithelium(thick arrow), lamina propria(thin arrow)(H&E, X:200). Ursodeoxycholic acid treated group(C):showing normal colon (H&E, X:100); Probiotic treated group(D): showing normal colonic tissue(H&E, X:100). Colitis group(E): showing distorted crypt(*), hyperplasia in epithelial cells with loss of goblet cell (thin arrow), ulcerated epithelium (thick arrow)(H&E, X: 200);(F): inflammation in mucosal layer(I), congested, dilated blood vessel(thick arrow), edema(thin arrow)(H&E, X: 100)(G): higher magnification of boxed area from pervious figure of colon tissues from colitis treated group showing leukocytic inflammatory cells in mucosal layer(blue arrow), congested, dilated blood vessel(thick arrow), edema(thin arrow)(H&E, X: 400). Colitis group treated with Ursodeoxycholic acid(H): showing lymphoid aggregates (arrow), intact crypts(H&E, X:100). Colitis group treated with probiotic(I): showing intact colon architecture(H&E, X:100). Colitis group treated with Ursodeoxycholic acid and probiotic(J): showing intact colon architecture(H&E, X:100).

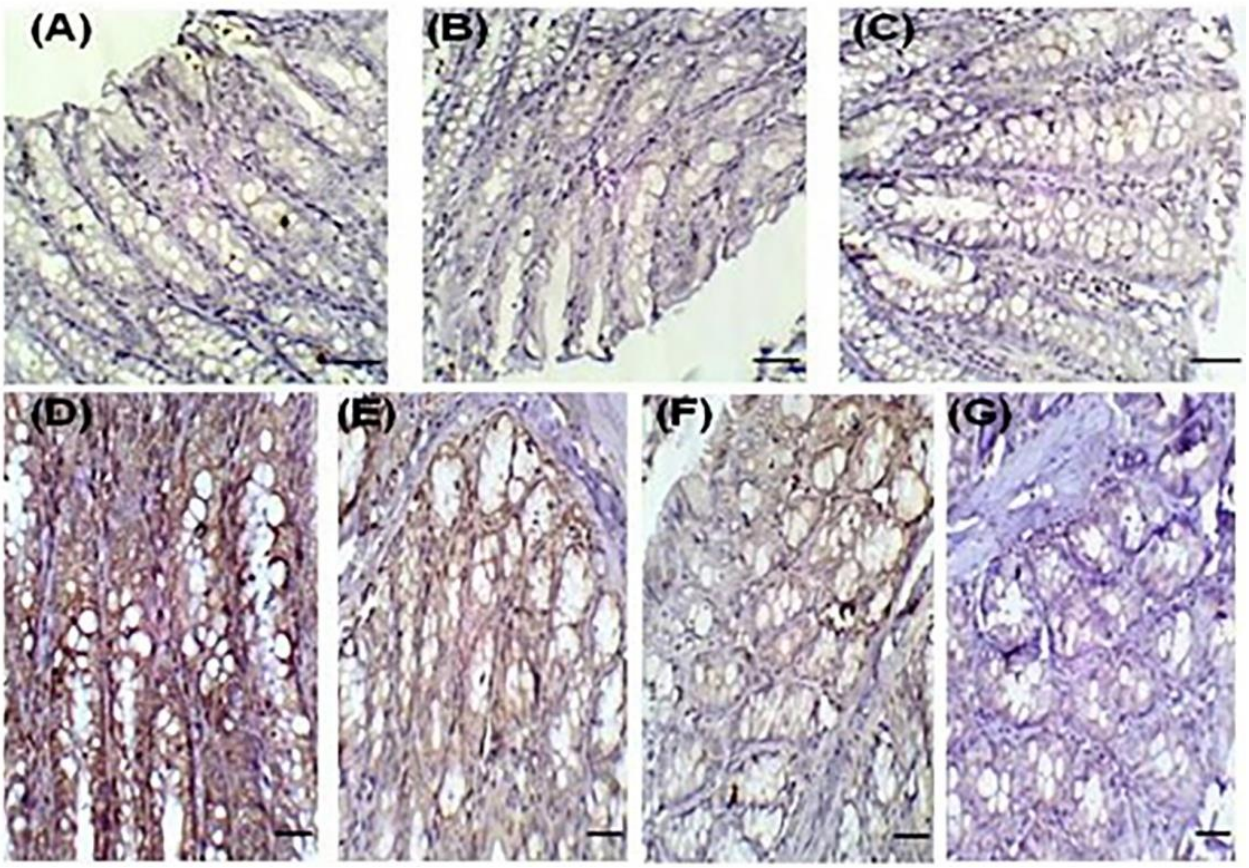

Fig. (5): IL-6 Immunohistochemical photomicrograph of colon tissues from (A)control group showing nearly –ve immunoexpression of IL-6; (B)Ursodeoxycholic acid group showing nearly –ve immunoexpression of IL-6; (C)Probiotic group showing nearly –ve immunoexpression of IL-6; (D)Immunoreactive IL6 antibody are observed in colonic mucosa and the lamina propria of acetic acid induced ulcerative colitis; (E)Ursodeoxycholic acid treated colitis group showing mild to moderate immunoexpression of IL-6; (F)Probiotic treated colitis group showing nearly –ve immunoexpression of IL-6; (G)Combined Ursodeoxycholic acid and Probiotic treated colitis group showing mild immunoexpression of IL-6(x:100).

1. Discussion

Ulcerative colitis(UC)is an incurable, chronic inflammatory bowel disease(IBD)that is characterized by idiopathic inflammation of the innermost lining of the colon and rectum[25] and is considered as a cytokine-mediated disease[26]. Acetic acid-induced colitis is an animal model of colitis that closely mimics human IBD in terms of the aetiology, morphological characteristics, and inflammatory markers that contribute to this kind of inflammation[27]. Mucosal ulceration, inflammation, bleeding, and weight loss in the colon are examples of colonic alterations[28]. Infiltration of the colonic tissues with inflammatory cells, increased inflammatory mediators release, disruption of the colonic barrier, and formation of reactive oxygen species(ROS) are all common features in UC disease. [29]. All currently utilized methods for treating UC are linked to a variety of negative side effects when used repeatedly or may exhibit decreasing response[30]. Thus, there is a constant need for new medications that help manage this complex illness.

Recently, great attention has been given to Ursodeoxycholic acid(UDCA), a physiological component present in human bile that showed a protective effect in the animal models of chemically induced colonic inflammation[31]. Probiotics are meant to have a variety of positive health effects whether taken orally or topically administered to the body. In order to maintain gut microbiota balance, boost gut barrier functions, and enhance host immune responses, probiotic bacteria are essential. Probiotic supplementation is thus becoming a therapeutic approach of great attention to reduce chronic inflammation caused by UC and enhance patients' quality of life[32].

In the current research, body weight significantly increased in the UC group that contradicts a plethora of studies[33,34]. The main cause of excess body weight may be attributed to the reduction of physical activity consequently in UC[35]. Moreover, a change in the distribution of adipose tissue may be the cause of weight gain that may lead to the accumulation of extra amounts of body fat[35] in intestinal homeostasis and inflammation

Administration of UDCA showed a significant increase of body weight while this finding is consistent with Van denBossche *et al.*(2017)[36]which could be attributed to bile acid's attenuation of intestinal microbiota control that plays a crucial role in the pathogenesis of IBD[37,38]. Moreover, Laukens *et al.(*2014) attributed the UDCA action to its enhancing colonic epithelial restitution[39]. Meanwhile, the combination of UDCA and probiotics showed a significant enhancement and increase in body weight.

The increase in colon inflammatory index is considered an indicator of the severity of inflammation in colitis. These results come in an accordance with Kandhare

et al.(2012), and Gupta *et al.*, 2015[40,41], which could be attributed to the increase in tissue water content, inflammatory cell infiltration, severe tissue edema, necrosis, and goblet cell hyperplasia[42]. Meanwhile, the use of UDCA and/or probiotics improves the severity of the colon to near-control levels, indicating that both treatments have good repairing effects.

Macroscopic examination results showed a marked visible thickening of the colon wall and hemorrhagic erosions, ulceration, and edema which is a hallmark of human UC and come in a harmony with previous studies[43,44].These observations could be due to an unbalanced response of the mucosal immune system toward bacterial antigens[45]. The conversion of acetic acid to a protonated state, which diffused into the epithelium and subsequently dissociated to produce protons, causes processes including localized inflammation, desquamation, and loss of mucosal integrity that result in epithelial damage and ulcers[42], this causes intracellular architecture to become more acidic, which triggers IBD[46].

In contrast, the treatment with UDCA and or probiotics significantly alleviated the total macroscopic damage scoring and number of colon ulcers of the colon. The intestinal anti-inflammatory action can be linked to the suppression of macrophage and neutrophil infiltration, highlighting the significance of these cells in the inflammatory process[47].

The levels of GSH were dramatically reduced, while MDA and MPO were upregulated by induction of acetic acid[33,47].The modulation of the results reflects the greatest potency of UDCA. Duboc *et al.(*2013) explained the secondary bile acids' synthesis from the primary is impaired in IBD patients[48]. It is believed that unconjugated UDCA is rapidly conjugated with glycine and to a lesser extent with taurine, on its first pass through the liver. Meanwhile, self-secretion of antioxidant metabolites and reducing enzyme activities that regulate ROS generation are examples of potential antioxidant pathways for probiotic benefits[49,50].

IL-10 is an anti-inflammatory cytokine that may be used to treat UC[51]. Meanwhile, proinflammatory cytokine IL-17 is abundant in the irritated mucosa of IBD patients[52]**.** IL-17 has a significant role in the etiology of the disease[53]**.** CRP level is also related to inflammatory status. It appears to be the tip used test to monitor and detect inflammatory disorders and is correlated with their severity but it's not specific to any illness[54].

In this investigation, IL10 in the UC group showed a significant decrease while UDCA normalized IL10 level. Lorén *et al.*(2015) accounted the effect of UDCA on IL-10 actions on the intestinal epithelium, by regulating the barrier function via a mechanism that involves the phosphorylation of p38 mitogen-activated protein kinases (MAPK)[55]. This would be probably due to reducing the inflammation-induced intestinal epithelial cell stress, helping to restore homeostasis and to isolate the lamina propria from luminal antigens. Meanwhile, probiotic administration showed no significant increase in IL10 levels where the combination of UDCS with probiotic significantly improve the level of serum IL10 in ulcerative colitis rats. The fact is that not all probiotic strains act in the same manner and that their efficiency varies depending on the concentrations used and the method of administration[56].

As expected, the treatment with probiotics or UDCA individually or in combination resulted in a significant reduction in IL17 and CRP levels, indicating that the severity of colon injury was reduced. This may reflect the capacity of both probiotic and UDCA to prevent the elevation of cytokine levels as a result to the increase in permeability of the epithelial cells to macromolecules associated with intestinal inflammation[57] and prevention of epithelial apoptosis as an indication to the UDCA anti-inflammatory effects[47]respectively. Moreover, the administration of probiotics alone or in combination with UDCA reduced CRP levels to near-normal levels which comes in line with de Souza *et al.(*2007)and Rodríguez-Padilla *et al.(*2021)[58,59]. The achieved normalization of the CRP could be explained by the ability of probiotics to interact with the intestinal mucosa, decreasing the molecular production of pro-inflammatory substances, and thereby decreasing the capacity for migration of inflammatory cells to the lamina propria, such as lymphocytes, eosinophils, and plasma cells[60,61].

Histological examination demonstrated that UDCA and probiotics reduced inflammatory cell infiltration and epithelial damage, resulting in a lower overall inflammation score. These findings were supported by Lajczak-McGinley *et al.*(2020) and Eeckhaut *et al.*(2013)[47,62]. The UDCA effects may be attributed to the prevention of cytokine-induced epithelial apoptosis, promotion of barrier function, and having a protective effect in a mouse model of colonic inflammation[47]. The protective impact of this probiotic against tissue damage may be due to their ability to limit the influx of inflammatory cells into the intestinal mucosa[63].

IL-6 was found mainly distributed in the mucosa and submucosa layers of colons. The outcomes are in agreement with Alex *et al.(*2009)who reported a significantly elevated levels in IL-6when compared to controls[64]. The increased IL-6 suggests that it acts synergistically to induce B-cell differentiation[65]. Treatment with UDCA and or probiotics showed enhanced colon recovery and normalization after treatment. These results agreed with Ward *et al.*(2017) and Hegazy and El-Bedewy(2010)[57,66]. This enhancement revealed the potency of UDCA in preventing the development of mucosal inflammation, which is closely associated with the inhibition of epithelial apoptosis[67]suggesting that probiotic therapy controls the mucosal immune response by lowering mucosal neutrophil numbers and may also work by inhibiting T-cell activation.

5. conclusion.

In conclusion, both UDCA and/ or probiotics considerably decreased inflammatory cytokines levels, intestinal villus damage, and MPO activity. Thus, findings from the current study confirm and support evidence suggesting that UDCA and/or probiotics may be useful in the treatment of inflammatory disorders of the colon.

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